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Note

Resolution of ethylphenols following *in situ* reaction on a thin-layer plate with Fast Blue B Salt

IVAN M. JAKOVLJEVIC and RAFIK H. BISHARA
The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind. (U.S.A.)
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The ethylphenol isomers (*o*-, *m*- and *p*-) are quite useful starting materials and intermediates in many syntheses for pharmaceutical and agricultural chemicals. However, the presence of a minor quantity of one isomer in another, as an impurity, usually results in the formation of the corresponding positional impurity in the final product. The isomeric purity of the starting ethylphenol material is therefore very important.

The literature[1–6] on the thin-layer chromatography (TLC) of alkylphenols generally indicates that the *ortho*-isomer has the greatest mobility and is adequately resolved from the *meta*- and *para*-isomers. On the other hand, the latter two isomers have the same mobility and are poorly resolved from each other. The present paper describes a new method involving the *in situ* spraying of the three ethylphenol isomers at the point of application (POA) with Fast Blue B Salt and the subsequent separation of the derivatives.

EXPERIMENTAL

Reagents and solutions

All chemicals used were reagent grade: isopropyl ether, "Baker Analyzed" (J. T. Baker, Phillipsburg, N.J., U.S.A.); 1,2-dichloroethane, ACS (Fisher Scientific, Fair Lawn, N.J.; U.S.A.); methanol, ACS (Matheson, Coleman & Bell, Norwood, Ohio, U.S.A.); Fast Blue B Salt (Naphthanil diazo blue B) (K & K Lab. Plainview, N.Y., U.S.A.); *ortho*-ethylphenol (Eastman-Kodak, Rochester, N.Y., U.S.A.); *meta*-ethylphenol (Aldrich, Milwaukee, Wisc., U.S.A.); *para*-ethylphenol (Eastman-Kodak).

Spray reagent. A 1-g amount of Fast Blue B Salt is dissolved in 100 ml of a mixture of 10 ml water and 90 ml of methanol. After 10 min of sonication the mixture is filtered through Whatman No. 1 filter-paper. A fresh solution is prepared daily.

Developing solvent. A 100-ml volume of 1,2-Dichloroethane–isopropyl ether, (90:10) is poured into the developing tank 30 min before introducing the plate.

Apparatus

Pre-coated, 0.25-mm thick silica gel 60 F-254 plates (20 × 20 cm) were from E. Merck (Darmstadt, G.F.R.), rectangular developing glass tank (28 × 22 × 8 cm) from Brinkmann (Westbury, N.Y., U.S.A.), ultrasonic bath from Heat Systems-

Ultrasonics (Plainview, N.Y., U.S.A.), 1-μl Microcaps pipettes, Drummond type, from Ace Glass (Louisville, Ky., U.S.A.), and chromatographic spray bottles, Cat. No. 2753-J10, from A. H. Thomas (Philadelphia, Pa., U.S.A.).

Procedure

All three isomers are dissolved in separate vials at a concentration of 10 mg/ml. A mixture of equal volumes of the three isomer solutions is also prepared.

The POAs are marked at 2.5 cm from the bottom edge of the plate. The adsorbent layer is scored 2.5 cm below the top edge of the plate to allow for a solvent front of 15 cm. A 1-μl volume of each isomer solution is spotted on lanes 1, 2 and 4. On lane 3, 3 μl of the mixture solution is spotted. Using a sheet of blotting paper (21 $\times$ 15 cm) the upper part of the plate is covered at a line parallel to the POA and at a distance of 3.5 cm above them. The blotting paper is secured to the plate with two small spring clips (Fig. 1). The exposed part is sprayed with the Fast Blue B Salt solution in five applications so that the plate is visibly wet. An attempt should be made to prevent the formation of drops on the plate due to overspraying. The blotting paper is then removed and the wet plate is allowed to dry at room temperature for 30 min. The dried plate is developed to allow the solvent front to reach the scored line at the top in about 90 min. The developed plate is removed from the tank and the solvent is allowed to evaporate in a hood for 5–10 min. The spray reagent is lightly applied to the dried plate. The sprayed plate is heated in an oven at 105° for 5 min and then examined.

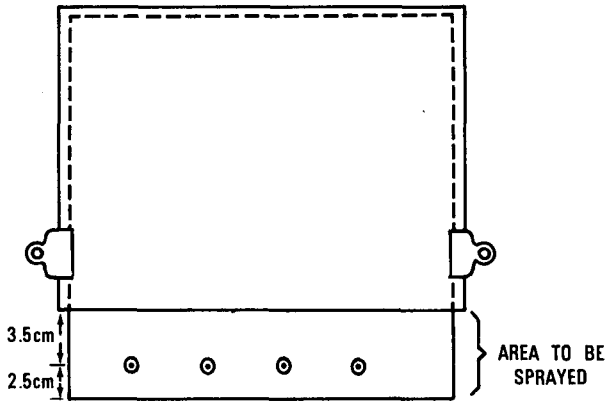

Fig. 1. Preparation of the spotted plate to be sprayed with Fast Blue B salt reagent (see text for details).

RESULTS AND DISCUSSION

The lower limit of detection for the three ethylphenols is about 1 μg. Fig. 2 shows the resolution of the *o*-, *m*- and *p*-ethylphenols in the conventional way (part A) and after the *in situ* reaction with Fast Blue B Salt (part B). Conventionally the *ortho*-alkylphenol derivatives are less strongly adsorbed than the *para*-isomers[7]. Bark and Graham[5] concluded that the increase in R_F value of the *ortho*-isomer is a result of a

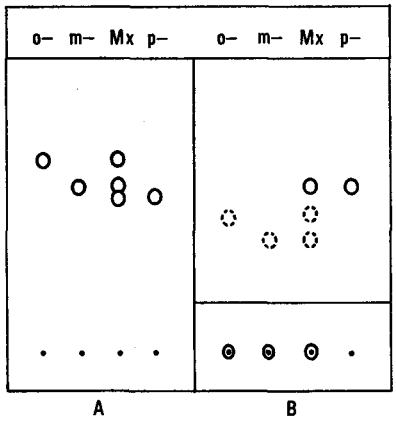

Fig. 2. Thin-layer chromatograms of ethylphenols: A; developed conventionally with 1,2-dichloroethane–isopropyl ether (90:10); B, first sprayed with Fast Blue B Salt and then developed with the same solvent as in A. 0 = *Ortho*; m = *meta*; p = *para*; Mx = mixture of the three ethylphenol isomers.

steric effect, the *o*-alkyl substituent reducing the availability of the phenolic group for bonding with the adsorbent surface. Because of the poor resolution of the *m*- and *p*-ethylphenols when conventionally developed (Fig. 2, part A), the idea of derivatizing the phenols was considered. The *in situ* derivatization reported in this paper achieves a good resolution between the two poorly resolved *m*- and *p*-ethylphenol isomers. To the best of our knowledge it represents the first *in situ* derivatization of phenols before their chromatography. However, many workers have derivatized the isomeric phenols before spotting them on the plate. Fast Red Al salt[8] and Fast Violet salt[9] are examples of reagents used. In addition, phenols were coupled with diazotized *p*-nitroaniline[10] or *p*-nitrophenylazo dyes[11] prior to their chromatography. In those examples[8–11] as well as in the system reported here, the order of mobility is *p*-> *o*-> *m*-. Thus a better resolution between the *m*- and *p*-isomers is attained and is superior to the poor resolution of the non-derivatized isomers. *Ortho*- and *meta*-isomers undergo some changes after reaction with Fast Blue B Salt reagent, hence their R_F values are reduced from 0.69 to 0.50 for *ortho*-, and from 0.61 to 0.43 for *meta*-isomer.

Spotting the single phenols or their mixture after being treated according to Knappe and Rohdewald[8] with Fast Blue B Salt prior to application on the TLC plate gave the same mobilities as those for the non-treated isomers. This indicated that no

TABLE I

MOBILITIES AND COLOR RESPONSES OF ETHYLPHENOLS

Isomers	R_F	*Derivatized with Fast Blue B Salt*	
		R_F	*Color response*
o-Ethylphenol	0.69	0.50	violet
m-Ethylphenol	0.61	0.43	violet
p-Ethylphenol	0.57	0.60	orange

derivatives were formed which was further confirmed by the lack of difference in the UV spectra for the treated and non-treated phenols. It may be concluded that the silica gel is an essential catalyst for the *in situ* derivatization of the phenols and for their subsequent separation.

The R_F values of the ethylphenols and their derivatives with Fast Blue B Salt as well as their color response are reported in Table I.

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